REMARKS

Entry of the foregoing amendments are respectfully requested.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By:_____
Teresa Stanek Rea
Registration No. 30,427

P.O. Box 1404
Alexandria, VA 22313-1404
Tel.: (703) 836-6620

Filed: March 20, 2002

defined by a portion of retina ESTs that is greater than 30% of the total. One of the 1241 entries meeting these criteria, Hs.60673, contained EST sequences from the 5'- and 3'-ends of two nearly identical cDNA clones isolated from the Soares retina N2b4HR cDNA library (ze39a04, ze32b03) **(http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html.)** Reverse transcription (RT)-PCR using oligonucleotides (SEQ ID NOS.: 46-47) A128F (5'-CTC ACA TCC TTC TCA GCC-3') and A128R (5'-GTG GAA TGT CAG GGA AAT C-3'), priming to sequences in the 5' reads of the cDNA clones, amplified a 193 bp transcript in retinal RNA but not in various other adult human tissues tested.

Inspection of the sequence of genomic clone NH0309N08 (GenBank Acc. No. AC007279) harbouring EST sequences from Hs.60673 revealed significant alignments with further ESTs derived from retina cDNA clones (ze27h05, ze30f10, zf58a06, ys72e09). On the basis of this additional cDNA sequence information, oligonucleotide primers (SEQ ID NOS.: 48-51) A128F3 (5'-TGA CTG CCT CCA GGA ATT-3'), A128aF (5'-TTA CGA AAT GAA TGG GCG-3'), A128aR (5'-AGG CTC TAG GTC CAT GAC-3') and A128R3 (5'-ATG TGA AAT CTG CGA AAG G-3') were designed and used to amplify retinal RNA in RT-PCR assays. The RT-PCR fragments were completely sequenced with walking primer technology on a ABI 310 automated sequencer (Perkin Elmer, Norwalk, USA) using the ABI PRISM Ready Reaction Sequencing Kit (Perkin Elmer, Norwalk, USA). Assembly of the overlapping 1375 bp A128F3/A128aR- and the 786 bp A128aF/R3-amplified cDNA fragments as well as 414 bp of 5' end sequence and 42 bp of the 3' end sequence of cDNA clone ze27h05 yielded a 2435 bp transcript with a conserved polyadenylation signal at nucleotide position 2416 bp. It should be noted that this full length transcript does not include the 5' end EST sequences of cDNA clones ze39a04 and ze32b03 (Hs.60673) which most likely have been derived from incompletely spliced mRNA precursor molecules.

The full length 2435 bp cDNA contains an open reading frame (ORF) of 1980 bp with a first potential *in frame* translation initiation codon, ATG, starting 69 nucleotides downstream (see Seq. ID No. 1). Therefore, the protein predicted from the ORF consists of 637 amino acid residues, resulting in a calculated molecular mass of 72.8 kDa and an isoelectric point of 5.4.

(B) Expression analysis

RT-PCR analysis using oligonucleotide primers (SEQ ID NO.: 52) A128F4 (5'-CGT GCC ATG ACT GAG TAC-3') and A128aR (sequence described above) identified an 844 bp product in human retina. No PCR amplification was observed in cerebellum, brain stem, liver, lung, heart, thymus, placenta, uterus, prostate, retinal pigment epithelium (rpe) and kidney. Northern blot analysis was performed with total RNA isolated using the guanidinium thiocyanate method (Chomczynski and Sacchi, Anal.Biochem. 162 (1987), 156-159). Each lane containing 10 mg of total RNA from temporal cortex, muscle, retina and liver was electrophoretically separated in the presence of formaldehyde. A 327 bp DNA fragment from the 3' untranslated region (UTR) was obtained by PCR amplification of genomic DNA with primer pair (SEQ ID NO.: 53) A128F6 (5'-AAC TGC AGT GGG TAC CAG-3')/A126R6 (sequence described above) and was used as a probe for filter hybridization in 0.5 mM sodium phosphate buffer, pH 7.2; 7% SDS, 1 mM EDTA at 58°C (Church and Gilbert, PNAS USA 81 (1984), 1991-1995). A single 3.8 kb transcript was identified exclusively in retina. The results of our expression analysis provide evidence that MPP4 is specific to the human retina. **(Figure 1).**

(C) Genomic organization and chromosomal location of MPP4

The publically accessible UniGene dataset, release no. 113, was searched for human EST clusters consisting of ESTs exclusively derived from retina cDNA libraries or for EST clusters with an enrichment of retina ESTs, defined by a portion of retina ESTs that is greater than 30% of the total. One of the 1241 entries meeting these criteria, Hs.60473, contained approximately 350 bp of high quality EST sequences from the 3'-ends of two cDNA clones (ze34f06, ze37g05) isolated from the Soares retina N2b4HR cDNA library. The approximately 280 bp high quality EST sequences of the 5'-end of the cDNA clones available at the dbEST database (http://www2.ncbi.nlm.nih.gov/dbST/dbest_query.html) do not overlap with the corresponding 3'end ESTs.

To isolate further cDNA clones representing this gene, a retina lambda-TriplEx2 cDNA library was screened with a radio-labeled 199 bp DNA fragment obtained by PCR amplification of genomic DNA with primers (SEQ ID NOS.: 54-55) A129F (5'-TCT GAG CCT AGA GGA TAC C-3') and A129R (5'-GAT CTC AGA GGC AGG TTG-3'). Fourteen positive clones with inserts ranging from 0.5 to 1.6 kb were isolated and sequenced with walking primer technology on an ABI 310 automated sequencer (Perkin Elmer, Norwalk, USA) using the ABI PRISM Ready Reaction Sequencing Kit (Perkin Elmer, Norwalk, USA)

To isolate the complete 5'-end of the cDNA the technique of 5'-RACE (rapid amplification of cDNA ends) was used (Frohman et al. PNAS USA 85 (1988), 8998-9002). First strand cDNA synthesis was primed using the gene-specific antisense oligonucleotide A129R. Following cDNA synthesis, the first strand product was purified from unincorporated dNTPs and remaining primers A129R. A homopolymeric tail was then added to the 3' end of the cDNA using terminal deoxynucleotidyl transferase (TdT) and dCTP. PCR amplification was accomplished using Taq DNA polymerase, the nested gene-specific primer (SEQ ID NO.: 56) A129R5 (5'-TGC TGT GAA GAT TGG AGA TC -3') that anneals to a site located within the cDNA molecule, and a deoxyinosine-containing abridged

anchor primer (SEQ ID NO.:57), AAP (5'-GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG-3') provided by Life Technologies, Rockville, USA To increase the quantity of the specific cDNA product the original PCR was re-amplified using the abridged universal amplification primer (SEQ ID NO.:58), AUAP (5'-GGC CAC GCG TCG ACT AGT AC-3') provided by GIBCO Life Technologies, and a second nested gene-specific primer (SEQ ID NO.:59) A129R4 (5'- AGC TTG AAG TGG CTA AAG TC-3'). Sequencing of the obtained PCR product using primer A129R4 did not reveal further upstream sequence suggesting that the identified cDNA sequence encompasses the complete 5' sequences starting from the transcription start site of the transcript.

Assembly of the cDNA sequences yielded a 1190 bp cDNA sequence which contains an open reading frame (ORF) of 638 bp with a first potential *in frame* translation initiation codon, ATG, starting 47 nucleotides downstream (Seq. ID No. 26-28). The encoded putative protein consists of 196 amino acid residues and has a calculated molecular mass of 22.3 kDa and an isoelectric point of 9.26.

Comparison of 14 different cDNA sequences revealed the presence of a single nucleotide polymorphism (C/G) at position 143 bp causing the amino acid substitution isoleucine to methionine at codon 32 of the putative protein sequence.

(B) Expression analysis

Reverse transcription-PCR analysis using oligonucleotide primer pairs A129F/A129R and A129F3 (SEQ ID NO.:60) (5'-TGA TCT CCA ATC TTC ACA GC-3')/A129R identified a specific 199 bp and 244 bp cDNA fragment in human retina only (Figure 2). No PCR amplification was observed in human cerebellum, liver, lung, heart, placenta, thymus and kidney. Northern blot analysis

was performed as was performed as described in Example 1. A 244 bp cDNA fragment from the 5' region was used as a probe for filter hybridization in 0.5 mM sodium phosphate buffer, pH 7.2; 7% SDS, 1 mM EDTA at 58°C. Two transcripts of about 0.85 and 1.20 kb were identified exclusively in retina (Figure 2).

(C) Genomic organization and chromosomal location of C7orf9

To determine the exon/intron structure of C7orf9, the 1190 bp cDNA sequence was aligned to the complete sequence of genomic BAC clone CTB-136N17 (GenBank Acc. No. AC004129) using the BLASTN program at NCBI. A total of 3 exons were identified with the putative translation start codon ATG located in exon 1 and the termination codon TAA in exon 3 (Seq. ID No. 26-28).

This genomic sequence of BAC clone CTB-136N17 contains DNA marker stSG51683 which has been mapped to the D7S2493-D7S529 interval on chromosome 7p15-p21 by screening the Genebridge4 radiation hybrid panel (http://www.ncbi.nlm.nih.gov/genome/seq).

(D) Nucleotide and protein database analyses

The cDNA sequence of C7orf9 was subjected to homology searches using the BLASTN program at Baylor College of Medicine (BCM)and revealed 100 % sequence identity between the coding region of C7orf9 and the human mRNA for RFamide-related peptide precursor (GenBank accession number AB040290). Therefore, the putative translation product of C7orf9 is identical to the RFamide-related peptide precursor (GenBank accession number BAB17674). The analysis for specific motifs using the integration tool for the signature-recognition methods in InterPro at the European Bioinformatics Institute. revealed that amino acids 99

screening and excised as plasmids from the phage vector following the instructions of the SMART[a] library kit manual (Clontech, Palo Alto, USA). In the case of the lambda-gt10 (SEQ ID NO.:63) cDNA library, one clone was isolated by PCR amplification. Primers A071F (described above) and lambda-gt10F (5'-AGC AAG TTC AGC CTG GTT AAG-3') were used to amplify the clone from a mixed phage lysate containing the positive clone. Additionally, 750 bp of F379 cDNA was amplified from retina cDNA using primer pair A071F (described above) and A071R2 (SEQ ID NO.:64) (5'- ATG TTC AGT CAG GCA GGG -3'). All cDNA library clones and PCR products were sequenced using the ABI PRISM Ready Reaction Sequencing Kit on an ABI 310 automated sequencer (Perkin Elmer, Norwalk, USA).

The 1188 bp full length consensus cDNA sequence of F379 (Seq.ID No.7) was determined from a compilation of the DNA sequences from the cDNA library clones, the PCR products and the ESTs of Hs.35493. An alignment of these sequences to the consensus cDNA sequence of F379 revealed that there were single base pair variations. These single base pair changes are summarized in Table 1. The full length consensus cDNA contained a putative open reading frame (ORF) of 85 amino acids (Seq. ID No. 31), starting at 347 bases from the most 5' end of the full length consensus cDNA. The single base changes in the cDNA do not truncate the putative ORF by introducing a stop codon; rather, the variations cause amino acid substitutions or have no effect on the putative ORF (Table 1). The ORF contains Alu and MIR repetitive elements, which together account for 68 amino acids. The predicted protein has a calculated molecular mass of 9.2 KDa and an isoelectric point of 6.81.

Table 1: Single base variations in the cDNA sequence and their associated amino acid changes

Position from beginning of cDNA	Nucleotide Change	Amino Acid Change
325	G	n/a*
429	T	L
442	A	R
528	T	I
557	T	S
932	A	n/a*
971	C	n/a*
987	T	n/a*

* single base pair variation is located outside of putative ORF

(B) Expression analysis

Reverse transcription-polymerase chain reaction (RT-PCR) using oligonucleotides A071F and A071R, priming to sequences in the 5' reads of the cDNA clones, amplified a 328 bp transcript from human retina RNA but not from uterus, cerebellum, heart, liver or lung RNA. Furthermore, Northern blot analysis was performed as described in Example 1. A 219 bp DNA fragment from the 3' region of the gene was obtained by PCR amplification of genomic DNA with primer pair A071F3 (SEQ ID NO.:65) (5'- TTC TTG TCG GAT GCC CTC -3') and A071R2 (described above). This DNA fragment was used as a probe for filter hybridization in 0.5 mM sodium phosphate buffer, pH 7.2; 7% SDS, 1 mM EDTA at 58°C. A single transcript of about 1.1 kb was identified only in retina. The

results of the expression analysis show that F379 is found exclusively in retina (Figure 3). Furthermore, the size of the transcript detected by Northern blot correlates to the size of the full length cDNA consensus sequence (1188 bp).

(C) Genomic organization and chromosomal location of F379

To determine the exon/intron structure of F379, the 1188 bp consensus cDNA sequence was aligned to the finished and unfinished genomic sequences using the BLASTN program at NCBI. The complete cDNA sequence of F379 aligned to genomic clones from different chromosomes, including chromosome 19 (LLNLR-222A1), chromosome 22 (RP11-395L14), chromosome 2 (RP11-559H14), chromosome 21 (RP11-34P13), chromosome 10 (RP11-438F6), chromosome 12 (RP11-598F7), and chromosome 9 (RP11-142M1). Partial alignments were also found to genomic clones from chromosome 15 (15qtel_c184at3), chromosome 12 (12PTEL057, 12PTEL055, RPCI11-55L14) and chromosome 19 (CTD-2102P23). These alignments identified three exons ranging from 205 bp to 621 bp. The putative translation start codon ATG is located in exon 1 and the termination codon TGA is located in exon 3.

PCR-based screening of two different human/rodent somatic cell hybrid DNA mapping panels also indicated the multicopy nature of F379. A commercial human/rodent somatic cell hybrid mapping panel (Mapping Panel 2 from Coriell Institute for Medical Research, Camden, USA) was screened with primer set A071F (described above) and A071R (described above), yielding a 328 bp product in cell line DNA containing chromosomes 2, 3, 6, 9, 12, 15, 19, and 20. Based on this result, gene names D2F379S1E, D3F379S2E, D6F379S3E, D9F379S4E, D12F379S5E, D15F379S6E, D19F379S7E, and D20F379S8E were assigned to chromosomes 2, 3, 6, 9, 12, 15, 19, and 20, respectively by the Genome Database (http://www.gdb.org/). The multi-chromosomal location of

Example 4: C12orf7

(A) Isolation of C12orf7 cDNA

The publicly accessible UniGene dataset, release no. 113, was searched for human EST clusters consisting of ESTs exclusively derived from retina cDNA libraries or for EST clusters with an enrichment of retina ESTs, defined by a portion of retina ESTs that is greater than 30% of the total. One of the 1241 entries meeting these criteria, Hs.28411, contained 10 EST sequences. Eight ESTs represent the 5'- and 3'-ends of four cDNA clones isolated from the Soares retina N2b4HR cDNA library (zf50g06, ze44g08, yt72c07, zf52h05) and two represent the 3'-ends of two cDNA clones isolated from the Soares placenta Nb2HP cDNA library (yi08f03.s1, yi75a07.s1).

To identify the full length cDNA transcript of C12orf7, a lambda-gt10 retina cDNA library was probed with a alpha^{32}P-dCTP-labeled 863 bp fragment obtained by PCR amplification of cDNA clone zf50g06 using primer pair (SEQ ID NOS.:66-67) A038F3 (5'-CGG AAC CGC TGT GAG TGC-3') and A038F (5'-TAG GCA GAG GTG GAT GGG-3'). The inserts of eleven positive clones were sequenced with walking primer technology using the ABI PRISM Ready Reaction Sequencing Kit on an ABI 310 automated sequencer (Perkin Elmer, Norwalk, USA).

Compilation of the 11 cDNA sequences revealed two different cDNA species. One cDNA molecule consists of 1428 bp, the second cDNA sequence contains an insertion of 30 bp at nucleotide position 549. To isolate the complete 5'-end of the cDNA the technique of 5'-RACE (rapid amplification of cDNA ends) was used as described in Example 2 except that first strand cDNA synthesis was primed with the gene-specific antisense oligonucleotide A038F and PCR amplification was accomplished using the gene-specific primer (Seq ID No.: 68) A038R3 (5'-GGC

CAC TCG GGC TTG TAG-3') and a second nested gene-specific primer (SEQ ID NO.:69) A038R4 (5'-GTG CAA TGC CAG CTC TTC-3'). Sequencing of the obtained PCR product using primer A038R4 revealed an additional 86 bp of 5' sequence. Assembly of the 5'-RACE sequence and the cDNA sequences obtained from the cDNA clones yielded a 1514 (Seq. ID No. 35) and a 1544 bp transcript (Seq. ID No. 36).

Comparison of the cDNA sequences revealed the presence of two single nucleotide polymorphisms at position 40 bp (A/T) and 88 bp (C/T) of Seq. ID No. 35 and 36.

Both cDNA variants contain the same putative open reading frame (ORF) encoding a 345 amino acid (aa) (Seq. ID No. 37) and a 355 aa (Seq. ID No. 38) protein. The putative proteins share the same potential *in frame* initiation codon, ATG, located 154 nucleotides downstream of the most 5' cDNA sequence. The putative protein sequences No. 11a and No. 11b have a calculated molecular mass of 37.1 kD and 38.0 kD and an isoelectric point of 5.59 and 5.49, respectively.

(B) Expression analysis

Reverse transcription-PCR using oligonucleotides A038F and A038R (SEQ ID NO.:70) (5'-TGC CAA GCT GTT AGT GCC-3'), priming to the 3' end of the cDNA sequence, amplified a 231 bp cDNA fragment from human retina RNA but not from human brain, heart, liver, lung or uterus RNA. RT-PCR using primers A038F4 (SEQ ID NO.:71) (5'-CAT GCT ACC ACG GCT TCC-3') and A038R3 amplified a 379 bp and 409 bp fragment from human retina RNA but not from human cerebellum, heart, kidney, liver, lung, placenta or thymus RNA (example in Figure 4).